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I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PS 1532 for a patent by H A MILTON HOLDINGS PTY LTD as filed on 04 April 2002.

WITNESS my hand this
Fifteenth day of April 2003

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PATENTS ACT 1990

PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

"NOVEL ANTI-BACTERIAL COMPOSITIONS"

This invention is described in the following statement:

BACKGROUND TO THE INVENTION

The present invention relates to novel anti-bacterial compositions, being toothpastes, dentifrices, mouthwashes, chewing gum or lozenges. The principal components of these toothpastes, dentifrices, mouthwashes, chewing gum or lozenges are (a) chlorhexidine or a salt thereof, such as chlorhexidine digluconate (commonly known as chlorhexidine gluconate), chlorhexidine diacetate or chlorhexidine dihydrochloride; (b) a zinc salt, such as zinc gluconate; (c) masking and/or flavouring agents, such as neohesperidine dihydrochalcone, saccharin and its salts, methyl salicylate and natural flavour oils such as mint; (d) suitable surfactants, such as macrogol ethers and zwitterionic betaine esters; and (e) other conventional ingredients of dental products, including preservatives, thickeners etc.

The toothpastes, dentifrices, mouthwashes, chewing gum or lozenges of the present invention may be used in the treatment of oral health problems, such as dental plaque, gingivitis and dental calculus, or as part of everyday oral hygiene practice.

Dental plaque is a complex mass, consisting mainly of bacteria that colonise the dental pellicle, the metabolic products of those bacteria, and other cellular material (epithelial cells and leukocytes). Dental plaque is the main etiological factor responsible for caries and periodontal diseases. The number and nature of the bacteria change continuously as the plaque develops, and different sites in the mouth may host different bacterial populations. The mode of attachment or aggregation of much of the oral bacteria is unclear. However, salivary aggregation, direct inter-species attachment, secretion of extra-cellular polysaccharides, physical entrapment of organisms, and presence of site-specific receptors are important factors.

The structure of plaque is soft and readily disrupted. The plaque structure derives from the properties of bacterial cell walls, cell wall polymers, and electrostatic bonding based on the presence of divalent cations such as calcium. Gingivitis is

caused when bacteria begin to grow at the gingival margin, generating toxins that cause inflammation. Gingivitis may be recognised by the gums becoming red and puffy, bleeding of the gums when they are subjected to minor trauma as caused by a toothbrush or flossing, and persistent bad breath. Unless treated, gingivitis can progress to periodontal disease. Although the progress from gingivitis to periodontal disease is not fully understood, it is believed that the process may follow the following stages:

- bacterial growth at the gingival margin;
- inflammation and bleeding of the gums;
- formation of gingival pockets (sulci);
- deepening of the gingival pockets to form periodontal pockets;
- trapping of plaque micro-organisms, debris and other material in these pockets;
- degenerative changes in surrounding connective tissue, caused by cellular and
- fluid exudates;
- widening of periodontal pockets, causing loosening of the teeth; and
- receding of the gums, thus exposing the roots of the teeth and causing
- discomfort.

Dental calculus is calcified plaque. Once considered to be the primary cause of periodontal disease through irritation, calculus is now considered to be of secondary importance. Nevertheless, calculus can be viewed as being the substrate on which further plaque can form.

Supragingival calculus arises from nucleation and subsequent crystallisation of calcium phosphate in plaque.

The aim of the present invention is to produce a toothpaste, dentifrice, mouthwash, chewing gum or lozenge which is pleasant tasting and has a good mouth-feel, is

low staining, cleans teeth and significantly reduces plaque build-up.

An ideal anti-plaque agent would inhibit bacterial adhesion to oral surfaces, disrupt pre-formed bacterial masses such as plaque, and maintain its effect for a long period of time.

SUMMARY OF THE INVENTION

Chlorhexidine is the most effective chemical anti-plaque agent currently available. It has anti-microbial activity against Gram-positive and Gram-negative bacteria, yeast and fungi.

Chlorhexidine is a particularly effective agent in the treatment of oral health problems. Animal and human studies have demonstrated that chlorhexidine in a mouthwash can effectively inhibit formation of dental plaque and gingival disease.

The mechanism of action of chlorhexidine is multifaceted:

- It interferes with bacterial absorption to teeth;
- disrupts established plaque;
- absorbs onto mucous membranes and teeth, desorbing from oral surfaces over a long period of time;
- has a long-lasting action, with residual anti-bacterial effects as long as 24 hours after application;
- is effective against a wide range of Gram-positive and Gram-negative bacteria; and
- leads to a general reduction of salivary microflora.

Chlorhexidine's exceptional anti-plaque activity can be attributed to its ability to adsorb onto dental surfaces and desorb therefrom gradually, providing, in effect, a timed release of the anti-microbial agent.

The anti-microbial properties of the chlorhexidine or salt thereof, which is the

active ingredient of the toothpastes, dentifrices, mouthwashes, chewing gum and lozenges of the present invention, is believed to derive from the following:

At high concentrations, it acts as a detergent, acting on cell membranes and causing loss of cytoplasmic constituents; at concentrations equal to or below the Minimum Inhibitory Concentration (MIC), it inhibits membrane transportation, metabolism and the activity of membrane-bound ATPase and various other enzymes.

However, there are serious problems in using chlorhexidine in oral hygiene products, such as toothpastes, dentifrices, mouthwashes, chewing gum and lozenges. Although considered safe for oral use, chlorhexidine is not widely used in toothpastes, dentifrices, mouthwashes, chewing gum and lozenges because it:

- stains teeth, the tongue and oral mucosa,
- has an exceptionally bitter taste, and
- is bio-inactivated in the presence of a range of common toothpaste ingredients.

The mechanism of staining is not well understood, but is known to be influenced by factors in the diet (such as tea, coffee or red wine) or personal habits (such as smoking).

Until now, attempts to develop a chlorhexidine toothpaste that is

- bioactive in terms of anti-plaque and anti-gingival effects,
- does not cause unacceptable staining, and
- has an acceptable flavour and mouth-feel

have not been successful.

It has previously been possible to overcome one or two of the problems listed above, but not all three. For instance, the use of zinc or other methods to prevent staining have been reported. However, the product either has failed to taste

satisfactory or has been bio-inactivated or both. Similarly, a pleasant tasting product may cause unacceptable staining, be bio-inactivated or both, and a bio-active product may cause staining or have unacceptable taste characteristics.

The present invention has surprisingly, through the use of a unique combination of ingredients, addressed all three problems associated with the use of chlorhexidine in oral hygiene products. The oral composition of the present invention may be a solution of ingredients, such as a mouthwash; a semi-solid product, such as a toothpaste or gel dentifrice; chewing gum; or a solid lozenge.

Accordingly, the toothpaste, dentifrice, mouthwash, chewing gum or lozenge of the present invention comprises (a) chlorhexidine or a salt thereof, such as chlorhexidine digluconate (commonly known as chlorhexidine gluconate), chlorhexidine diacetate or chlorhexidine dihydrochloride; (b) a zinc salt, such as zinc gluconate; (c) masking and/or flavouring agents such as neohesperidine dihydrochalcone, saccharin and its salts, methyl salicylate and natural flavour oils such as mint; (d) suitable surfactants, such as macrogol ethers and zwitterionic betaine esters; and (e) other conventional ingredients of dental products, including preservatives, thickeners etc. Other zinc salts or gluconate salts may also be present.

It is also known that chlorhexidine does not impede the formation of calculus and may even promote its formation, but the addition of zinc salts reduces this. Although the mechanism is not well understood, it has been postulated in US Patent No. 4,522,806 that an antibacterial agent alone kills or retards the growth of bacterial plaque but, in the absence of zinc ions, the remaining dead bacteria mineralize to form calculus. The zinc ions act as crystallization inhibitors and interfere with nucleation, whereas antibacterial agents interfere with bacterial colonization of the plaque. When zinc ions and an antibacterial agent are used together, the zinc ions co-act to keep the plaque porous and more penetrable by the antibacterial agent. Zinc salts therefore may enhance the plaque inhibition of anti-microbial agents in some instances. It is also known that soluble zinc salts have anti-microbial activity when present in sufficient concentration. It is possible the

zinc ions and chlorhexidine compete with each other, both seeking the same receptor sites on the bacteria, and this has been supported by the observation that pretreatment by zinc ions reduces the antiplaque effectiveness of chlorhexidine. It has also been reported that the staining effect of chlorhexidine is reduced in the presence of zinc salts and, surprisingly, in a clinical trial using the composition described in this specification, we have found that the chlorhexidine staining is reduced and is easier to remove in the presence of zinc gluconate and that it appears similar to normal food staining. Accordingly, normal brushing with the composition removes a significant portion of the staining due to chlorhexidine.

Surfactants are used in the compositions of the present invention to achieve increased foaming action and maintain the flavours in dispersion. The surfactant material most commonly used in toothpaste is anionic; however, this class of surfactant is incompatible with chlorhexidine salts. According to one aspect of the present invention, a novel combination of non-ionic and zwitterionic surfactants is used, which combination provides good foaming of the toothpaste and does not bio-inactivate the chlorhexidine. Typical non-ionic surfactants include glycerol esters of fatty acids, sorbitan esters of fatty acids, macrogol esters (polyethylene or polyoxyethylene glycol esters), macrogol ethers (condensation products of polyethylene glycol and fatty alcohols, usually cetyl or cetylstearyl alcohol), polysorbates, and polyoxyethylene/polyoxypropylene/polyoxyethylene block co-polymers of general formula $HO(CH_2CH_2O)_x(CH(CH_3)CH_2O)_y(CH_2CH_2O)_zH$. Typical zwitterionic esters include amino acids of general structure $RNHCH_2CH_2COOH$, betaines of general structure. $RN^+(CH_3)_2CH_2COO^-$ and alkylamido alkyl amines having general formulae of, for example, $RCONHCH_2CH_2N^+(CH_2COO^-)HCH_2CH_2OH$ and $RCONHCH_2CH_2N^+(CH_2\ CH_2COO^-)HCH_2CH_2OCH_2CH_2COOH$. A suitable combination is of the macrogol ether, ceteareth 30, and cocamidopropyl betaine, eg in the ratio of 2.4:1 by weight, which may, for instance, constitute a total of 1.7% w/w of the toothpaste, although the maximum content of the combination may be as high as 10% w/w and as low as 0.1% w/w.

Examples of suitable flavouring constituents include, but are not limited to, flavouring oils, eg oil of spearmint, peppermint, wintergreen, sassafras, clove, sage, eucalyptus, cinnamon, lemon, and orange, and methyl salicylate. The use of masking agents is necessary since conventional sweetening agents such as xylitol and sorbitol are not sufficient to cover the bitter chlorhexidine taste. The taste is persistent and previous attempts to mask it in oral preparations containing chlorhexidine have had variable success. Artificial sweetening agents such as saccharin and its salts and cyclamate and its salts have been used. It is known, however, that saccharin or salts thereof will complex with chlorhexidine causing precipitation and bio-inactivation of the chlorhexidine. Also, the masking effect of such artificial sweeteners is transient, lasting only a short time while the masking compound is present in the mouth. Thus, saccharin on its own is ineffective, and it has been shown that neohesperidin dihydrochalcone, being of medium intensity sweetness on its own, is also ineffective.

Surprisingly, it has been discovered that the combination of saccharin or a salt thereof, preferably saccharin sodium, with neohesperidine dihydrochalcone in certain ratios did not bio-inactivate or precipitate the chlorhexidine, while providing a long-lasting masking effect of the bitter chlorhexidine taste. When combined with one or more of the flavouring oils described above, the product produced was pleasant tasting with little after-taste. Suitable masking agents include saccharin and its salts and neohesperidin dihydrochalcone. Suitably, flavour and sweetening agents may each or together comprise from about 0.1% to 5% w/w or more of the preparation. The use of a combination of relatively low levels of saccharin or a salt thereof with a long-lasting sweetener such as neohesperidine dihydrochalcone, as a sweetening/masking agent in chlorhexidine formulations, forms a novel aspect of the present invention.

When the oral composition is substantially semi-solid or pasty in character, such as a toothpaste or gel, the dentifrice vehicle may contain a dentally acceptable water insoluble abrasive material such as sodium metaphosphate, potassium metaphosphate, tricalcium phosphate, dihydrated dicalcium phosphate, anhydrous

dicalcium phosphate, calcium pyrophosphate, calcium carbonate, aluminum silicate, hydrated alumina, calcined alumina, silica, bentonite, and mixtures thereof.

The abrasive material is generally present in the paste or gel composition in weight concentrations of about 5% to about 60% by weight, preferably about 10% to about 30% in a gel and about 5% to about 60% in a paste.

Toothpastes, as well as gel dentifrices, typically contain a natural or synthetic thickener or gelling agent in proportions of about 0.1 to about 10% by weight, preferably about 0.5 to about 5% by weight. Suitable thickeners or gelling agents include Irish moss, iota-carrageenan, kappa-carrageenan, gum tragacanth, starch, polyvinylpyrrolidone, hydroxyethyl propyl cellulose, hydroxybutyl methyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose, sodium carboxymethyl cellulose, Veegum and other silica-derived materials.

A further aspect of the invention relates to a method of incorporating chlorhexidine or a salt thereof in a toothpaste, dentifrice, mouthwash, chewing gum or lozenge, so as to form a product having acceptable characteristics with regard to bioactivity, taste and staining of teeth, wherein the chlorhexidine or salt thereof is accompanied by a zinc salt, and the toothpaste, dentifrice, mouthwash, chewing gum or lozenge further comprises: masking and/or flavouring agents such as neohesperidine dihydrochalcone, saccharin and its salts, methyl salicylate and natural flavour oils such as mint; suitable surfactants; and other conventional ingredients of dental products, including preservatives, thickeners etc. Preferably, the formulation comprises chlorhexidine gluconate, or another gluconate salt.

Fluoride materials may also be included in the oral compositions of the present invention to provide an anti-caries effect. Suitable such materials are inorganic fluoride salts, preferably soluble alkali metal fluoride salts, for example sodium fluoride, potassium fluoride, sodium monofluorophosphate and sodium hexafluorosilicate. The fluoride-providing salt is generally present in the oral composition at a concentration of about 0.0005 to about 3.0% by weight.

Where the oral composition of the present invention is substantially liquid in character, such as a mouthwash or rinse, the vehicle is typically a water-alcohol mixture. Generally, the weight ratio of water to alcohol is in the range of from about 3:1 to 10:1, and preferably about 4:1 to about 6:1. The alcohol is a non-toxic alcohol such as ethanol or isopropanol. A humectant, such as glycerine, sorbitol or an alkylene glycol such as polyethylene glycol or preferably propylene glycol, may be present in amount of about 10-30% by weight. Mouthwashes or rinses typically contain about 50-85% by weight of water, about 0 to 20% by weight of a non-toxic alcohol and about 10-40% by weight of the humectant.

DETAILED DESCRIPTION OF THE INVENTION: EXAMPLES

The invention will now be further described with respect to the following examples, which are illustrative but not restrictive of the present invention.

EXAMPLE 2: Toothpaste Formulation

The formulation is similar to that of Example 1, except that an appropriate amount of sodium fluoride is added to the aqueous phase B).

EXAMPLE 3: Clinical Studies

Introduction

A study was carried out in the Department of Dentistry, University of Adelaide, in conjunction with Hamilton Laboratories, to test the effect of a new formulation of a toothpaste containing chlorhexidine (CHX) gluconate (as per Example 1) on dental plaque formation. Slurries of the toothpastes being tested were prepared and used as rinses, with subjects abstaining from mechanical plaque control for 4 days. The slurry method is used because mechanical plaque control alone, performed by a skilled person, would reduce dental plaque formation, with or without the use of a toothpaste or antibacterial agent. A commercial Oral Rinse formulation from Zila Pty Ltd (Peridex, containing 0.12% w/w chlorhexidine gluconate solution) was chosen as a positive control because of its well documented ability to suppress plaque formation.

Methods

Subjects

Volunteers participating in the study were non-smokers, in good general health and included both women and men between 20 and 55 years of age. They did not use any mouthwash or toothpaste containing chlorhexidine as part of their routine oral hygiene practices. The subjects were chosen because they formed large amounts of plaque in a previous pilot study.

The following protocol was used:

The trial was conducted over a period of several weeks, with the subjects attending a dental clinic on the morning of the first day for a prophylaxis. The subjects were then provided with one of four blinded samples to be used as a rinse on the evening of day 1; twice daily, morning and evening, for days 2, 3, 4; and once in the morning of day 5. Mechanical plaque control was not allowed during this period. On the morning of day 5, the subjects again attended the clinic, the plaque on their teeth was disclosed by the application of a dye, and a score in the range of 0-5 (where 0 was no plaque, 5 was maximum plaque) for individual teeth was determined. The teeth were then photographed and given a prophylaxis. The subjects were then allowed to resume their habitual plaque control for a week while resting, to allow for washout of any chlorhexidine they may have encountered. On the first day of the third week, the whole process was repeated, with the subject receiving a different preparation to trial, and so on until all four samples had been tested. One of the randomized samples was a chlorhexidine mouthwash used as a control. This control has been widely used in clinical trials examining the anti-plaque activity of toothpastes as it provides a consistent, very high anti-plaque activity, but it is a simple non-viscous solution without the complexity of taste, foaming and physical characteristics expected of a consumer product.

At no time did the subject or the examiner know which preparation was being used. The four preparations were randomly distributed amongst the subjects in such a manner that all subjects used all four preparations but in varying order. The key was held in a secure location until the completion of the trial.

The protocol is summarized in Table 1.

TABLE 1

Monday	Friday
Prophylaxis.	Plaque disclosing.
Cease mechanical plaque control.	Photograph anterior teeth (2/3 magnification).
Commence rinsing 2x daily for 60 secs with 3g toothpaste slurry.	Prophylaxis.
	Recommence habitual plaque control.

The samples used were:

A- Hamilton CHX toothpaste (0.6% w/w) with Fluoride

B- Hamilton CHX toothpaste (0.6% w/w)

C- Chlorhexidine Gluconate solution (0.12% w/w solution) (Control)

D- Placebo

Determination of plaque coverage of teeth

The teeth were examined after 4 days, and a dye was applied to highlight the plaque. A photograph of the teeth was taken as a record.

Results

Assessment of plaque area

The plaque indexes for the four preparations were determined. The control mouthwash, Preparation C, provided the anticipated level of plaque protection. There was a mathematically significant difference between Preparation B (0.6% chlorhexidine gluconate toothpaste) and the placebo (Preparation D), and also between Preparation A (0.6% chlorhexidine gluconate toothpaste with fluoride) and the placebo (Preparation D) – Preparations A and B both performed significantly better than the placebo. Although plaque suppression by Preparation B was better than for Preparation A, Preparation A still provided acceptable results.

While the present invention has been described in terms of preferred embodiments in order to facilitate better understanding of the invention, it should be appreciated that various modifications can be made without departing from the principles of the invention. Therefore, the invention should be understood to include all such modifications within its scope.

Dated this 4th day of April, 2002.

H A MILTON HOLDINGS PTY LTD
By its Patent Attorneys
MADDERNS